**International Journal of Zoology Studies**
**ISSN: 2455-7269; Impact Factor: RJIF 5.14**
**Received: 04-10-2020; Accepted: 20-10-2020; Published: 12-11-2020**
**www.zoologyjournals.com**
**Volume 5; Issue 6; 2020; Page No. 24-29**

# Heavy metals bioaccumulation in the food chain of the silkworm *Bombyx mori* L

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## Abstract

Anthropogenic and natural sources can be considered as the major sources of heavy metal release into environment. Highly toxic and reactive concentrations from such metals proposed to enter soils and groundwater, bioaccumulation in food webs, and adversely affect biota. Silkworm is the most important natural manufacture. Introduced study aims to investigate the effect of heavy metals accumulation in the harvestable parts of the plants like leaves is the route to the tropic levels in the food chain of the silkworm (*Bombyx mori*). The biological parameters were assessed to show the impact of heavy metals pollution on the silkworm life cycle and productivity. The results of the X–rays' analysis of soil and plant leaves samples showed that there were significant differences between the concentrations of heavy metals in different soils. The higher concentrations were Zn > Cr > Cu > Pb > Cd > Sr, from the site I, and site II and this may be due to the irrigation by contaminated water with heavy metals, but the lower concentrations were found in the control soil which irrigated by treated water (tap water). The higher heavy metals concentrations in the leaves were Zn > Cu >Pb > Cr > Cd, from the site I, and site II. But the lower concentrations were found in control leaves sample. Moreover, the pollution in the silkworm food chain lead to general reduction in the growth rate of the larval stages, the weight of cocoon, cocooning percentage, the Effective Rate of Rearing, and the silk yield. It also results in increasing the mortality rate of larvae. It is recommended that heavy metals pollution in the food chain of silkworms should be strictly monitored.

**Keywords:** heavy metals, bioaccumulation, mulberry, silkworm, translocation

## 1. Introduction

Sericulture is the discipline of silkworm rearing for raw silk processing. Silkworm, *Bombyx mori L.* feeds on the mulberry (*Morus spp.*) leaves for its growth and development. The production parameters of silkworms depend on the larval nutrition and the quality of mulberry leaves. Heavy metals contamination can be assumed as a main environmental soil problem [1, 2, 3].

Some heavy metals are necessary for the plant growth and for animal and human health. If these heavy metals found in higher concentrations, they became toxic [4]**.** The heavy metals are necessary for plant metabolism and development, but the higher concentration of heavy metals in the plant or the soil lead to toxicity and inhibition of plant processes [5, 6, 7]. There are toxic effects of heavy metals on mulberry plants. At a lower soil content of Cd, there is no harmful effect on mulberry plant growth. At a higher soil content of Cd, there was a reduction in the leaves. At a very high content of Cd in soil, the plants growth is reduced [8]. Cr has an effect on the growth of plants that shows alterations in the germination process, like the growth of roots, stems and leaves [9]. Toxic effect of food contamination with cobalt Co (II) on *B. mori L.*

larval stage is observed on the larvae length in each instar, the growth and the mortality rate get worse [10]. The entry of zinc (II) into the food chain of *B. mori L.* from mulberry plants is responsible for toxic effects on the life cycle [11]. Furthermore, Silver Nanoparticles effect on the growth of silkworm *B. mori.* At high concentrations, the rate of growth increases leading to silkworm death [12]. Zinc affects on the reproductive system of silkworm *B. mori L.* Mulberry leaves with (Zn) that are fed to the larvae increase the quality parameters of cocoons like higher shell cocoon ratio, silk-body ratio, raw silk percentage, and increases the fecundity of silk moth [13].

Environmental pollution is the worst problem of the world now a days.

The goal of this research was planned to study the accumulation of heavy metals in the food chain of *B. mori* and its impact on the larvae growth, the cocoon weight, cocoon shell, length of silk thread, and silk production.

## 2. Materials and methods

The present study was carried out in laboratory of Entomology, Zoology Department, Faculty of Science, Mansoura University during spring and autumn seasons (2019) to investigate the effect of heavy metals on the food chain of mulberry silkworms (*B. mori L.*) and its silk product.

### 2.1 Mulberry leaves

The mulberry (*M. Alba*) leaves were collected and introduced as food to silkworms' larvae (*B. mori L.*) from three sites: Garden of faculty of Agriculture, Mansoura University.

(Site I) -Delta Company of fertilizers and chemical industries (http://www.delta fertilizer.com/delta/). (Site II)-Garden of faculty of Science, Mansoura University. (Control)

### 2.2 Preparing of silkworm's culture

Eggs of *B. mori L.* of local hybrid were obtained from the Sericulture Research Department, Plant Protection Research Institute of the Agricultural Research, Mansoura, Egypt.

### 2.3 Rearing room

Stands, rearing equipment and the floor were disinfected using bleaching powder solution (5%), and then the whole room was disinfected by formalin solution with 3% concentration. The rearing room was kept airtight for 24 hours and then the room was kept open for fresh air and used for rearing. Polythene sheet as bottom and cover were used.

### 2.4 Rearing technique

Rearing of silkworm was carried out under laboratory conditions. The eggs of silkworm *B. mori L.* were incubated at 25± 10ºC and 75 to 80% relative humidity. The eggs were surrounded by wilted rubber strips and covered with paraffin paper to maintain optimum relative humidity. The eggs were put in black box for 24hrs at blue egg stage, and then were subjected to diffused light for uniform hatching. The newly hatched larvae were fed with chopped mulberry leaves from the different sites. Plastic net was used for removing the faeces and dried food.

### 2.5 Experimental design

The newly hatched *B. mori L.* larvae were divided into three groups. The 1st group of larvae fed on mulberry leaves from the garden of Agriculture Faculty, the 2nd group fed on mulberry leaves from the Delta Company of fertilizers and chemical industries. Every group was divided into three subgroups (three replicates) in the 4th instar- each replicate was fifty larvae.

Chicken eggs cartons were used as montages for cocoons spinning (Zannoon and Omera) [14]. The cocoons were harvested seven days later. Fresh cocoons of each treatment were taken for measuring the cocoon indices. Fresh cocoon, cocoon shell and pupae were weighed for all treatments; another group of the cocoons was used for the biological studies. After emergence, each couple was placed inside a paper box for mating and laying eggs.

### 2.6 Analysis of heavy metals in soil and mulberry leaves

#### 2.6.1 Preparation of plant samples

The harvested leaves were wrapped in polythene bag and taken to the laboratory. The leaves washed in water, air dried and then placed in Oven at 80°C for drying of samples. After oven drying samples were cooled, ground and stored in plastic bags. The dried leaves were ashed in a furnace of 500 $^{0}$C for 4 h, in a cool muffle furnace, ashed samples were allowed to cool and then stored in airtight plastic containers ready for elemental analysis.

#### 2.6.2 Preparation of soil samples

1. Soil samples were collected from 0-15 cm. soil samples were taken to laboratory.
2. Samples were dried at 80$^{0}$ C. ground with wooden roller and sieved through 2 mm stainless steel sieve.

#### 2.6.3 Digesting and determination of metals concentration in the solid samples

1. Samples were dried in the oven at 105ºC till constant weight.
2. Weigh 2gm from each sample.
3. Transfer a weight dry sample to a beaker.
4. In a hood, add a little amount of deionized water and 5 10 ml of conc. $HNO_3$ and cover with a watch glass.
5. Heat the sample gently on a hot plate.
6. Continue heating and adding conc. $HNO_3$ as necessary until digestion is complete as shown by a light-colored sample.
7. Do not let sample dry during digestion.
8. Wash beaker walls and watch glass cover with deionized water and then filter.
9. Finally, complete the sample to 50 ml and mix thoroughly.
10. The metal concentration was measured using inductive coupled plasma optical emission spectrometry (Agilent ICP-OES 5100, Australia) according to standard methods for the examination of water and wastewater (APHA, 2017) [15].

### 2.7 X–ray analysis of soil and plant leaves samples

X-ray fluorescence spectroscopy was employed using a Portable X-MET8000 expert XRF analyzer that delivers the performance needed for fast alloy and soil accurate identification was used to identify surface soil composition under study.

A layer of about 3-5 cm of the soil surface was removed from the three sites. Measurement of heavy metals concentration was performed in a triplicate sample position and data was recorded as an average measurement.

Mulberry leaves were removed from the three sites, after drying the measurement of heavy metals concentration was performed and data was recorded.

### 2.8 Cocoon characters

Fresh cocoon weight (g): Ten cocoons were taken from each replication on the fifth day of mounting and each one was weighted, then the average was calculated.

Cocoon shell weight (g): The ten cocoons were cut to remove the pupae and the cocoon shell weight was recorded and the average was calculated.

Cocooning percentage (%).

$$\text{Cocoon shell ratio \%} = \frac{\text{Weight of cocoon shell}}{\text{Weight of fresh cocoon}} \times 100$$

Effective rate of rearing (%): The number of cocoons harvested at the end of rearing was recorded and ERR percentage was calculated by using formula:

$$\text{ERR (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms brushed}} \times 100$$

Silk filament length (m): Five cocoons from each replication were taken and each one was reeled using Eprovette and silk filament length was recorded by the formula:

L = R x 1.125m. Where,

L= Length of the silk filament (m)

R= Number of revolutions.

1.125m= Circumference of the Eprouvette.

$$\text{Raw silk ratio \% (Renditta)} = \frac{\text{Weight of dry raw silk}}{\text{Weight ofdry cocoon}} \times 100$$

Denier (D): Is the weight in grams of 9000 m of yarn per filament, which is indicative of the quality of the silk. The sizes of reelable filaments were calculated according to Tanaka [16] formula

$$D = \frac{\text{Weight of silk filament (g)}}{\text{Length of silk filament (m)}} \text{ x } 9000$$

### 2.9 Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA). Comparisons of means of non-emergence adults were made with the Duncans Multiple Range Test (Costat Software, 2004) [17].

## 4. Results & Discussion

### 4.1 Heavy metals concentration in soil samples

Data in Fig (1) showed the concentration of some heavy metals in soil samples that were planted by mulberry trees (*M. Alba*). During the spring and autumn rearing seasons of 2019.

The results showed that there were significant differences between the concentrations of heavy metals in different soils.

The higher concentrations were Zn > Cr > Cu > Pb > Cd > Sr, from the site I, and site II and this may be due to the irrigation by contaminated water with heavy metals, but the lower concentrations were found in the control soil which irrigated by treated water (tap water). The results are in agreement with the findings of Shahid *et al.* [18] who reported that the increase in heavy metal terrestrial ecosystems' contamination through anthropogenic activities is a widespread and serious global problem due to their environmental and human implications.

**Table 1:** Heavy metals concentrations in soil samples from the Faculty of Agriculture, Mansura University (Site I) and Delta Company of fertilizers and chemical industries (Site II), compared to the control (C), by Inductive Coupled Plasma optical emission spectrometry (ICP) and XRF analysis

| Element (mg/Kg) | ICP analysis | | | XRF analysis | | |
|---|---|---|---|---|---|---|
| | C | Site I | Site II | C | Site I | Site II |
| Cd | 0.09 ± 0.11 | 0.05± 0.02 | 0.05± 0.05 | 0.02± 0.01 | 0.03± 0.01 | 0.03± 0.02 |
| Cr | 17.34 ± 0.02 | 62.50± 0.03 | 60.00± 1.15 | 16.22± 0.02 | 60.01± 0.02 | 55.52± 0.01 |
| Cu | 9.54 ± 0.02 | 35.00± 1.15 | 30.00± 2.00 | 11.02± 0.02 | 33.12± 0.02 | 32.12± 0.02 |
| Pb | 10.00± 1.15 | 27.50± 0.02 | 15.00± 1.15 | 9.86± 0.02 | 28.00± 1.15 | 17.05± 0.02 |
| Zn | 5.30 ± 0.02 | 175.00± 2.0 | 55.0± 2.00 | 13.45± 0.02 | 60.52± 0.01 | 50.06± 0.02 |
| Co | 0.85 ± 0.36 | 1.25± 0.02 | 1.50± 0.02 | 1.10± 0.02 | 1.15± 0.01 | 1.45± 0.02 |
| Se | 0.03 ± 0.02 | 0.05± 0.11 | 0.05± 0.01 | 0.01± 0.01 | 0.03± 0.01 | 0.03± 0.02 |
| L.S.D 0.05% | 0.40 | 0.76 | 1.08 | 0.01 | 0.38 | 0.01 |

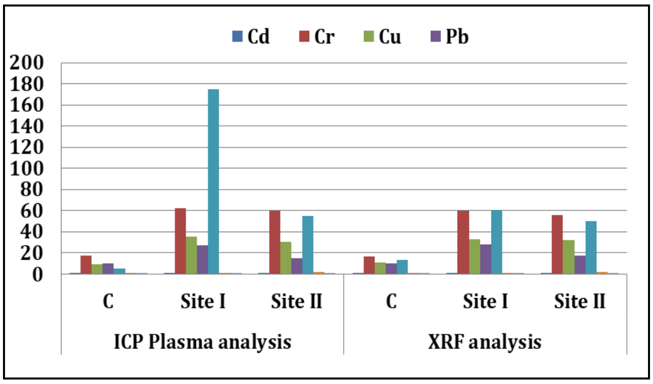

**Fig 1:** Heavy metals concentrations in soil samples from (Site I) and (Site II), compared to the control (C), by optical emission spectrometry (ICP) and XRF analysis

### 4.2 Heavy metals concentration in mulberry leaves samples

The heavy metals residue content in (*M. alba*) leaves that were varied significantly is shown by Fig (2). The higher heavy metals concentrations in the leaves were Zn > Cu >Pb > Cr > Cd, from the site I, and site II. But the lower concentrations were found in control leaves sample.

The results confirm the impact of the contaminated soil on the mulberry plant leaves and pollutants transferred to the basic level of the food chain. These results are with agreement of Shahid *et al.* [19] who noticed that the contamination of soil by heavy metals led to accumulation of these metals in crops that not only decrease crop productivity but act as a serious risk to animal and human health. Besides, to Pourrut *et al*. and Shahid *et al*. [20, 21] who proved that the major source of heavy metals in agricultural soil is the use of untreated wastewater (industrial and city wastewater) for plant irrigation.

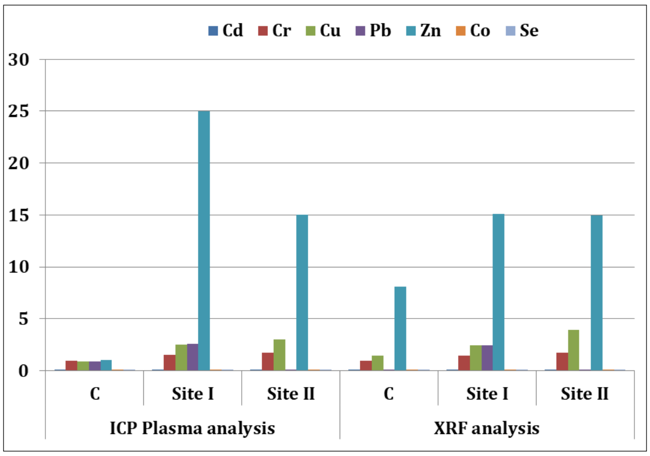

**Fig 2:** Heavy metals concentrations in mulberry leave samples from (Site I) and (Site II), compared to the control (C), by inductive coupled plasma optical emission spectrometry ICP and XRF

### 4.3.2 Cocoon parameters

The results in Table 3 showed the effect of heavy metals on the weight of cocoon, weight of cocoon shell, the rate of cocooning and the effective rate of rearing % (ERR) during spring and autumn seasons (2019). The weight of fresh cocoon, the weight of a single shell (cocoon without pupa), and the percentage of cocooning decreased in the cases of site I and site II, and this may be due to the increasing of heavy metals concentrations in the mulberry leaves. The percentages of ERR also decreased as well. These results in close similarity with Tariq A. Khan *et al.* [22] who estimated the effect of dust pollution on the silkworm larvae *B. mori L.* Feeding the larvae with extremely dust polluted leaves during late age showed a Significant reduction in the single cocoon, shell weights and shell ratio. Also, Kalai S. and

Vijaya Bhaskara [23] mentioned that feeding Silkworms chemical contaminated mulberry leaves by chemical pesticides lead to toxicity of silkworm, reducing the cocoon yield. Copper is one of the constituents of different fungicides that effects on the growth, reproduction, and survival of living organisms and silkworms. The best values of the weight of single cocoon, shell cocoon, the percentage of cocooning and ERR were at the control group that fed on mulberry leaves with lower content of heavy metals.

The effective rate of rearing was found to be good in the control group (97.41%) in spring season followed by site II (90.38 %) and (15.44%) in site I. These results are in accordance with those found by Younus M. Wani *et al* [24] investigating the role of mineral nutrition as zinc on the synthesis proteins in silkworms. The diet was provided with minerals, vitamins and trace elements.

Zinc increased the quality parameters of cocoons like higher shell cocoon ratio, silk-body ratio, raw silk percentage.

**Table 2:** Effect of heavy metals on the biological development of *B. mori* cocoon, from (Site I) and (Site II), compared to the control (C), during the season spring- autumn 2019.

| Study site | Spring | Autumn | Spring | Autumn | Spring | Autumn | Spring | Autumn |
|---|---|---|---|---|---|---|---|---|
| | Weight of single fresh cocoon (g) | | Weight of single cocoon shell (g) | | Cocooning | | Effective rate of Rearing (ERR %) | |
| C | 1.55±0.03 | 1.23±0.01 | 1.18±0.02 | 1.05±0.02 | 70.41±0.01 | 70.0±0.02 | 97.2±0.01 | 96.6±0.02 |
| Site I | 0.60±0.05 | 0.53±0.02 | 0.47±0.03 | 0.42±0.05 | 40.12±0.01 | 40.0±0.06 | 15.4±0.02 | 15.0±0.03 |
| Si te II | 0.89±0.02 | 0.84±0.05 | 0.72±0.04 | 0.71±0.01 | 66.43±0.02 | 66.1±0.04 | 90.3±0.03 | 89.2±0.04 |
| L.S.D 0.05% | 0.11 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.06 |

### 4.8 Effect of heavy metals on the different parameters of the silk yield of B. mori L

The results in Table (3) showed the relation between the accumulation of heavy metals in the body of silkworm larvae and the length (Fig 3.b), the weight of dry cocoon, Denier (Fig 3. A), Rendita (row silk ratio) (Fig 3.c) of silk thread during spring and autumn 2019. Obtained data revealed that *B. mori L.* was reared during spring lead to better results compared with rearing in the autumn season. The results are in agreement with the findings by Maryam *et al.* [25] who noticed that the spring season was better than autumn season during the rearing period. Also, Ghazy [26] agreed with these results.

The highest values of the weight, and denier of silk thread were estimated in the control group, followed by site I and site II (FIG 3A). The maximum length of silk thread (1407m) and the weight of dry cocoon (0.33g) were recorded in site II. These results are agreed to Kavitha *et al* [27] noticed that the economic parameters of both cocoon and silk (silk yield, shell weight, floss weight, floss-shell ratio, floss silk ratio, raw silk weight, renditta and denier) were significantly modulated under the influence of zinc. Similarly, to Bhaskar P.*et al.* [28] studied the negative effect of copper sulphate on the total protein, protease, and the level of free amino acids in silkworm. Silkworm fed with 15ppm treated copper sulphate leaves activated the amino acids contents in the silk gland of the *Eri* silkworm and that led to increasing the silk production.

**Table 3:** Effect of heavy metals on the different parameters of silk yield of *B. mori* L. from (Site I) and (Site II), compared to the control (C), during the season spring - autumn 2019

| Study site | Weight of dry cocoon (g) | | Weight of silk thread (g) | | Silk denier | | Length of silk thread (m) | | Raw silk ratio (Rendita) (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Spring | Autumn | Spring | Autumn | Spring | Autumn | Spring | Autumn | Spring | Autumn |
| C | 0.29±0.01 | 0.26±0.04 | 0.1±0.03 | 0.09±0.04 | 1.43±0.03 | 1.01±0.01 | 981.50±0.05 | 952.0±0.57 | 25.3±0.05 | 24.5±0.05 |
| Site I | 0.22±0.02 | 0.20±0.03 | 0.08±0.02 | 0.07±0.05 | 0.63±0.02 | 0.54±0.01 | 1175.0±0.57 | 1054.0±0.57 | 37.4±0.05 | 36.3±0.05 |
| Site II | 0.33±0.05 | 0.30±0.03 | 0.07±0.01 | 0.06±0.21 | 0.47±0.01 | 0.44±0.03 | 1407.0±0.57 | 1230.0±0.33 | 22.6±0.05 | 21.5±0.05 |
| L.S.D 0.05% | 0.01 | 0.09 | 0.06 | 0.42 | 0.01 | 0.01 | 1.63 | 1.76 | 0.19 | 0.19 |

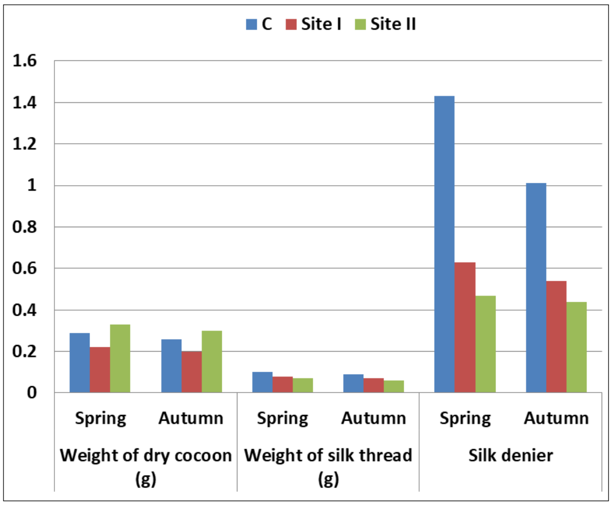

**Fig 3. A:** Effect of heavy metals on the different parameters of silk yields of *B. mori L.* from (Site I) and (Site II), compared to the control (C), during the season spring - autumn 2019.

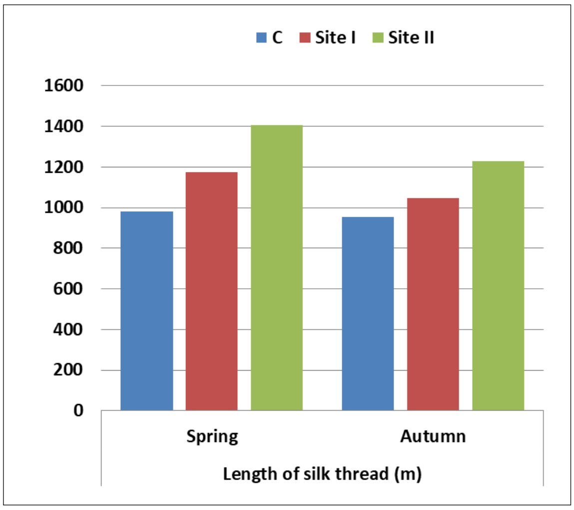

**Fig. 3. B:** Effect of heavy metals on the length of silk thread of *B. mori L.* from (Site I) and (Site II), compared to the control (C), during the season spring - autumn 2019.

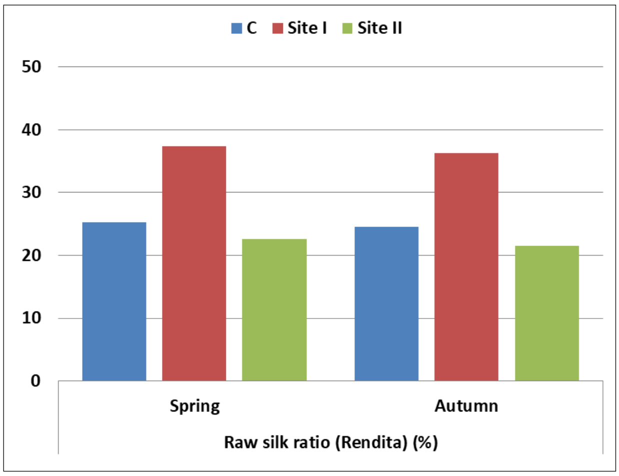

**Fig 3. C:** Effect of heavy metals on Raw silk ratio % of *B. mori* from (Site I) and (Site II), compared to the control (C), during the season spring - autumn 2019.

## 5. Conclusions

The present study conducted to assess the impact of bioaccumulation of heavy metals on the different biological parameters of *B. mori L.* during spring and autumn 2019.

Feeding silkworms with polluted mulberry leaves that contained high concentrations of heavy metals leads to reducing the growth of the larvae, the weight of cocoon, cocooning percentage, the Effective Rate of Rearing, and the silk yield. It also results in increasing the mortality rate of larvae. The harmful effects of heavy metals on the sericulture cannot be ruled out, so management strategies are required to ensure the sustainability of this vital industry. Our work suggests that heavy metals pollution in the food chain of silkworms should be strictly monitored.

## 6. Acknowledgment

Authors thanks the company of fertilizers “Delta Company of fertilizers and chemical industries (http://www.delta fertilizer.com/delta/). (Site II)” for giving the permission to collect samples from the manufacture area.

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